

20. Fish and Aquatic Life Impacts from Low pH and Dissolved Metals

METAL UPTAKE, TRANSFER, AND HAZARDS IN THE STREAM FOOD WEB OF THE UPPER ANIMAS RIVER WATERSHED, COLORADO

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Science for Watershed Decisions on Abandoned Mine Lands: Review of Preliminary Results, Denver, Colorado, February 4-5, 1998

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Streams in the upper Animas River watershed of Colorado drain a highly mineralized basin which has been extensively mined for gold and other heavy metals. The water quality, aquatic habitats, and stream biota of these streams are affected by acid drainage and metal contamination from hundreds of abandoned mines and from natural weathering of rocks and soil. We conducted a survey of metal concentrations in water, suspended colloids, sediment, and stream biota from the upper Animas River watershed. The objectives of this study were to examine relationships among metal concentrations in water, suspended colloids, sediment, and biota (periphyton, benthic invertebrates, and fish); transfer of metals among trophic levels; and potential hazards of metal toxicity to fish.

METAL BODY BURDEN AND BIOLOGICAL SENSORS AS ECOLOGICAL INDICATORS

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Quantitative laboratory and field investigations were integrated to link ecological metrics with metal body burden in sentinel aquatic organisms. The aim was to define organismal and molecular descriptors that will be useful in supplementing ecological assessments. To this end, the relationship of metal body burden to the presence and abundance of key taxa in impacted and unimpacted stream sectors was determined. Various organisms were evaluated to determine suitability as sentinels for monitoring metal exposure. Metal analyses were conducted on water, invertebrates, and fish tissue to quantify the amounts of cadmium, chromium, copper, lead, nickel, silver, and zinc present in each type of media. The study was conducted on Big Bayou Creek, KY. Results showed substantial differences in metal body burden values for different aquatic taxa, which appeared to correspond to differences in trophic relationships and assimilation of metals. Metal residues in stoneroller minnows provided an accurate means of identifying outfalls of bioavailable metals. Metal accumulation in this species was inversely proportional to ecosystem changes. The development and application of a novel in vitro metal biosensor are described, which responds to bioreactive metals entering across the apical surface of a reconstructed gill epithelium.

HEAVY METALS IN ORGANISMS OF THE RIVER GUADALQUIVIR ESTUARY: POSSIBLE INCIDENCE OF THE AZNALCOLLAR DISASTER

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Mollusks, fish, and crustaceans were collected in the Guadalquivir estuary in southwestern Spain following contamination due to a breached mine tailings pond, and heavy-metal concentrations were measured. Results showed that the spill affected *Crassostrea angulata* the most, due to the high zinc

contents, but cadmium and copper levels were also quite high. While metal levels in most estuarine organisms were higher at the impacted sites than at the reference sites, the data on background metal levels were insufficient to assess the full impact of the spill.

A STRATEGY FOR USE OF MULTIVARIATE METHODS OF ANALYSIS OF BENTHIC MACROINVERTEBRATE COMMUNITIES TO ASSESS MINE-RELATED ECOLOGICAL STRESS

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Analysis of the structure of the benthic macroinvertebrate community, consisting of the number of taxa and the relative distribution of total numbers, is a routine method of assessing the ecological quality of streams. Various multivariate statistical techniques, which assess the structural variability of the benthic macroinvertebrate community and the relationship of that variability with natural and anthropogenic environmental variables, have utility in separation of the various physical and chemical factors associated with impact. Multivariate methodology, while computationally complex, is statistically rigorous and better integrates physical, chemical, and biological data for more synthetic interpretation. Using the multivariate analysis technique, canonical correspondence analysis, sites in the upper Boulder River Basin, Montana, have been analyzed to determine which are the important environmental variables associated with variation in community structure.

SENSITIVITY TO ACIDIC PH IN BENTHIC INVERTEBRATE ASSEMBLAGES WITH DIFFERENT HISTORIES OF EXPOSURE TO METALS

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A number of studies have documented tolerance in aquatic communities exposed to contaminants, but few have examined costs of tolerance and potential community-level consequences. We assessed the effects of metals and acidic pH, a novel stressor, on communities from streams with different histories of metal pollution. Acidic pH was a novel stressor to communities in streams that are consistently circumneutral. Intact benthic invertebrate assemblages collected from metal-polluted sites on the Arkansas River (AR1, low levels of pollution, and AR5, greater levels of pollution) and a reference site on the Cache la Poudre River (PR), Colorado, were exposed to either metals (Zn, Cu, Cd) or pH 4.5 in stream microcosms. Multivariate analyses on a subset of taxa showed different patterns of response to metals and acidic pH among the 3 sites, which corresponded to exposure histories of the communities: PR assemblages were more sensitive to metals, whereas AR assemblages were more sensitive to acidic pH. These patterns were supported by analyses on specific characteristics of community structure. Exposure to metals significantly reduced abundance of mayfly taxa in PR ($p = 0.0041$, ANOVA, linear contrast), AR1 ($p = 0.0108$), and AR5 ($p = 0.0329$) assemblages and taxa abundance in PR ($p = 0.0225$) and AR1 ($p = 0.0469$) assemblages. Total invertebrate abundance also decreased in PR assemblages exposed to metals ($p = 0.0274$). The results suggested that greater metal tolerance within the Arkansas River communities was a result of assemblage-level differences in community structure and population-level differences in sensitivity among sites. In contrast, Arkansas River assemblages, especially those from AR5, were sensitive to acidic pH, which reduced invertebrate abundance in PR ($p = 0.0339$), AR1 ($p = 0.0284$), and AR5 ($p = 0.0062$) assemblages. Abundances of mayflies and mayfly taxa also were significantly lower in AR1 ($p = 0.0247$, $p = 0.0042$, respectively) and AR5 ($p < 0.0001$, $p = 0.0006$, respectively) assemblages exposed to acidic pH

but were not significantly altered in PR assemblages. These differences among sites in response to acidic pH resulted primarily from differences in community composition. For instance, the AR5 mayfly assemblage was dominated by *Baetis* spp., which were eliminated from all treatment streams. In contrast, less sensitive mayfly taxa (*Rhithrogena hageni* and *Ephemerella infrequens*) were abundant at stations AR1 and PR. Our results suggested that chronic metal pollution may have produced communities tolerant of metals but more sensitive to acidic pH.

THE TOXICITY OF IRON TO BROWN TROUT AND EFFECTS ON THE GILLS: A COMPARISON OF TWO GRADES OF IRON SULPHATE

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Iron added to reservoirs to reduce available phosphate levels and algal growth typically is an acidic ferric sulfate liquor by-product of titanium extraction and can be contaminated with trace metals. The potential toxicity of iron sulfate to brown trout, particularly in the gills, was studied. Fish were exposed to lethal and sublethal concentrations of iron as the commercial liquor and analar grade to determine acute toxicity. For the commercial liquor, the 96-h LC50 was 28 mg total iron/l, and for analar grade, it was 47 mg/l. Iron accumulated on the gills and caused gill damage for both grades at both concentrations. There was no evidence of iron uptake in gill tissues or of iron accumulation in fish plasma in exposed fish compared with controls. Iron exposure may disrupt the respiratory process via physical clogging of the gills.

EVALUATION OF THE RECOVERY OF FISH AND INVERTEBRATE COMMUNITIES FOLLOWING RECLAMATION OF A WATERSHED IMPACTED BY AN ABANDONED COAL SURFACE MINE

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U.S. Geological Survey Toxic Substances Hydrology Program: Proceedings of the Technical Meeting, 8-12 March 1999, Charleston, South Carolina. Volume 1: Contamination from Hard-Rock Mining

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A 5-yr study was conducted to measure the rates of recovery of fish and invertebrate communities following reclamation of a watershed impacted by an abandoned coal surface mine in Southwest Missouri. Quarterly monitoring of water quality information (pH, conductivity, and alkalinity) was conducted at 10 sites. Annual monitoring of biological (benthic invertebrate and fish community structure), physical (substrate grain size), and chemical (metals, pH, conductivity, and alkalinity) variables was conducted at 5 of the 10 sites. Prior to the reclamation effort the stream was nearly devoid of aquatic life above Hwy 2 for a distance of approximately 2 miles due to extremely low pH (<3) and elevated levels of calcium, magnesium, iron, zinc, aluminum, copper, strontium, boron, and cobalt. State water quality standards for zinc, copper, and cadmium were exceeded. Fish were present at reference sites (largemouth bass, white crappie, bluegill, minnows, and darters) but were absent at sites impacted by acid mine drainage within the project boundaries. Benthic invertebrates were similarly impacted. Reclamation activities were initiated late in 1991 and continued through 1995. Significant recovery of water quality, fish, and invertebrate communities were observed following the reclamation. Both chemical and biological approaches were useful in monitoring the recovery of the aquatic system following the watershed reclamation. The paper is available at <http://toxics.usgs.gov/pubs/wri99-4018/Volume1/index.html>

CHARACTERIZING THE AQUATIC HEALTH IN THE BOULDER RIVER WATERSHED, MONTANA

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U.S. Geological Survey Toxic Substances Hydrology Program: Proceedings of the Technical Meeting, 8-12 March 1999, Charleston, South Carolina. Volume 1: Contamination From Hard-Rock Mining

U.S. Geological Survey Water-Resources Investigation Report 99-4018A, p 55-58, 1999

The Boulder River and some of its tributaries receive direct effluent from abandoned mine adits and runoff from old tailings piles located in the basin. This biological assessment identified a pathway of metals exposure in the Boulder River Watershed as measured by concentrations of metals in biofilm (abiotic and biotic material on rock surfaces), invertebrates, and fish collected from the Boulder River and a select number of its tributaries. These data along with data from fishery population surveys are being used to assess the ecological health of the Boulder River and its tributaries. Preliminary data suggest that concentrations of arsenic, copper, cadmium, lead, and zinc are elevated to varying degrees in biological tissues collected from the Boulder River and its tributaries. Tissue damage in fish livers, as measured by an increase of products of lipid peroxidation, along with reductions in fish sizes and populations in lower Cataract Creek were also noted. Thus, exposure to metals may have resulted in a deterioration of fish health and a quantitative loss in fish populations in Cataract Creek. We also documented 100% mortality of fish placed in live containers in the upper sections of the Basin Creek and Cataract Creek and lower High Ore Creek.

DEVELOPMENTAL ABNORMALITIES AND DNA-PROTEIN CROSSLINKS IN SEA URCHIN EMBRYOS EXPOSED TO THREE METALS

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Embryos of the purple sea urchin, *Strongylocentrotus purpuratus*, were exposed to nickel, chromate, and arsenate, and developmental abnormalities and DNA-protein crosslinks were determined. A mammalian assay utilizing potassium chloride precipitation of proteins was adapted to separate free DNA and protein-crosslinked DNA in cellular extracts. The embryos were exposed to sublethal concentration ranges of each metal over a 48-h period, corresponding to developmental stages from early cleavage to prism. Results showed that the embryos exhibited impairment of both spicule and archenteron formation after exposure to metal concentrations that induced DNA-protein crosslinks. Both the developmental abnormalities and the DNA-protein crosslinks in the embryos increased in a concentration-dependent manner for all three metals, but exposure to arsenate was most toxic. Embryos exposed to Ni at different time points during development showed a pronounced difference in sensitivity depending on the developmental stage, with adverse effects more pronounced with earlier exposure.

INDUCED METAL TOLERANCE IN MICROBENTHIC COMMUNITIES FROM THREE LOWLAND RIVERS WITH DIFFERENT METAL LOADS

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The response of microbenthic communities to sustained metal stress was studied in three lowland rivers with different levels of pollution. Tolerance against zinc and cadmium was determined in short-term toxicity tests with microbenthic assemblages colonizing glass discs. Photosynthetic activity served as an endpoint in tests for algae, whereas for bacteria thymidine incorporation was determined. For bacterial

assemblages from unpolluted locations, zinc and cadmium values varied in short-term tests. Bacterial assemblages from the two most polluted sites were significantly more tolerant for zinc. Results indicated a shift in community composition toward pollution-adapted organisms when a threshold concentration of 1 μM zinc is exceeded. Although an increasing community tolerance was also indicated for algae, values for microbenthic algae from all sites exceeded in most cases the highest metal concentrations tested (Zn: 1,000 μM ; Cd: 320 μM). Since species composition of algal assemblages was found to change at much lower metal levels, it is concluded that short-term toxicity tests measuring photosynthesis inhibition do not reflect well the long-term effects of these metals. Toxic effects of metals on both algal and bacterial assemblages are attenuated by precipitation and complexing capacities of the biofilm.

IN VITRO DOSE-RESPONSE STUDY OF THE EFFECT OF CADMIUM ON EEL (*ANGUILLA* *ANGUILLA*) GILL Na^+/K^+ -ATPASE ACTIVITIES

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Ecotoxicology and Environmental Safety, Vol 28 No 1, p 43-52, Jun 1994

Eels, which migrate through waters having wide variations in salinity and a high likelihood of heavy metal contamination were used in an in vitro study of the effect of cadmium on the activity of sodium/potassium ATPase in the gills. Eels were acclimated to either fresh or salt water, exposed to various concentrations of Cd, and studied for effects on enzyme activity. At the highest freshwater and saltwater concentrations of Cd, decreases of 40 and 25%, respectively, were demonstrated in basal activity, as measured by magnesium ATPase activity. A dose-dependent inhibition of Na^+/K^+ ATPase activity was demonstrated by exposure to Cd. At a concentration of 146 nM, 50% of the activity was inhibited, as measured by two different techniques, for eels adapted to both saltwater and freshwater.

MANGANESE REMOVAL BY CHEMICAL AND MICROBIAL OXIDATION AND THE EFFECT ON BENTHIC MACROINVERTEBRATES AT A COAL MINE IN WAYNE COUNTY, WESTERN WEST VIRGINIA

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Proceedings, 18th Annual West Virginia Surface Mine Drainage Task Force Symposium, 15-16 April 1997, Morgantown, WV

Manganese removal from discharge waters is a serious problem in many states where Appalachian Basin coal is mined. We analyzed benthic macroinvertebrates and bacteria upstream and downstream from mining operations at the Queens Fork surface mining operations in Wayne County, western West Virginia, in order to assess the ecological effect of elevated instream Mn levels. Manganese data were also retrieved from the U.S. Geological Survey QWDATA database to compare mine wastewater Mn concentrations with those in other Wayne County surface and ground waters. The benthic macroinvertebrates included sensitive, facultative, and tolerant species. A small shift from more sensitive types upstream to more tolerant ones downstream is occurring throughout the ongoing benthic study. No statistically significant differences in abundance and number of taxa existed between the upstream and downstream localities. Rocks upstream and downstream from mining are coated black from Mn oxide precipitates. Glass microscope slides were placed in various sites to mimic rock surfaces and allow bacteria to colonize them for study. The major microbial taxon precipitating Mn upstream and downstream from mining was *Leptothrix discophora*, a species that is typical of natural settings having elevated Mn levels. Other unidentified Mn-fixing species dominated within the wastewater treatment ponds. Dissolved Mn concentrations in wells are higher than surface waters suggesting that many

streams in Wayne County are fed by ground waters carrying elevated concentrations of Mn. This paper is available at <http://energy.er.usgs.gov/products/papers/wvsmddf/index.htm>

THE EFFECT OF COPPER ON THE BLOOD CHEMISTRY OF CLARIAS GARIEPINUS (CLARIIDAE)

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Ecotoxicology and Environmental Safety, Vol 29 No 2, p 187-199, Nov 1994

The catfish, *Clarias gariepinus*, was exposed to copper concentrations simulating those found in the Olifants River of Kruger National Park, South Africa. Exposure to typical winter and summer concentrations of 0.085 and 0.05 mg/l, respectively, resulted in physiological responses as manifest in altered blood chemistry. Hyperglycemia, leucocytosis, and other pathological conditions were observed. While the fish physiologically adapted to this environmental stressor, this trend does not always reflect a state of normality.

COMPARISON OF BENTHIC DIATOM ASSEMBLAGES FROM STREAMS DRAINING ABANDONED AND RECLAIMED COAL MINES AND NONIMPACTED SITES

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We examined whether there was a particular group of diatoms specific to acid mine drainage (AMD) sites and/or reclaimed sites in streams in a coal-mining region of southeastern Ohio. Streams were initially placed into 5 categories: 1) stream receiving AMD from an unreclaimed site, 2) stream receiving drainage from a site reclaimed prior to a 1972 regulation, 3) stream receiving drainage from a site reclaimed between 1972 and 1982 under Ohio Revised Code (ORC) 1513, 4) stream receiving drainage from site reclaimed after 1982 under the Surface Mining Control and Reclamation Act (SMCRA), and 5) stream not impacted by AMD. The diatom flora from riffles in each system and environmental parameters (pH, conductance, metal concentrations [Al, Fe, Mn], current velocity, width, and depth) were examined to assess the recovery of reclamation sites from mining. Canonical correspondence analyses separated heavily impacted AMD streams from other sites. Total alkalinity and pH were highly correlated to the 1st axis, and SO₄, average depth, and temperature were influential in additional axes. Discriminant analyses of the diatom and environmental data sets were successful in assigning samples into 1 of the a priori stream categories (85% and 81.8% accuracy, respectively). AMD streams were characterized by a dominant flora of *Eunotia exigua* and *Frustulia rhomboides*. Streams that fluctuated between acidic and circumneutral pH (termed oscillating) had greater abundances of *Brachysira vitrea* than other study streams. Streams of intermediate water quality (i.e., reclaimed sites) were dominated by *Achnanthes minutissimum*. There was a predictable relationship between post-reclamation stream water quality and diatom assemblages, which may prove useful in assessment and management of reclamation efforts.

ROLE OF THE GILLS AND GILL CHLORIDE CELLS IN METAL UPTAKE IN THE FRESHWATER-ADAPTED RAINBOW TROUT, *ONCORHYNCHUS MYKISS*

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Canadian Journal of Fisheries and Aquatic Science, Vol 51 No 11, p 2482-2492, Nov 1994

Fish absorb contaminant metals from the external environment primarily through their gills. A series of tests were conducted to investigate the metal uptake characteristics of freshwater-adapted rainbow

trout, *Oncorhynchus mykiss*. Fish were exposed for a 12-hour period to waters containing elevated concentrations of isotopes of cadmium, zinc, and copper. Gill tissues were then sampled and analyzed against other tissues. Gills from the fish concentrated metals 3- to 11-fold. Gill tissues contained 38-50% of the total metal burden. These tissues only sustained small amounts of epithelial damage. The levels of chloride cell surface area were varied, but produced no significant changes in metal uptake. Results of analyses of liver and kidney cells are included.